

## *Conservation and Utilization of the Genetic Resources of Apples, Grapes, and Tart Cherries*

### **ARS LOCATION:**

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### **PROJECT OBJECTIVES:**

1. Strategically expand the genetic diversity in genebank collections and the quality and quantity of associated information for priority cold hardy grapes and their wild relatives.
2. Conserve and regenerate priority cold hardy grape genetic resources efficiently and effectively, and distribute samples and associated information worldwide.
3. Strategically characterize (“genotype”) and evaluate (‘phenotype’) priority cold hardy grape genetic resources for molecular markers and highly heritable horticultural and morphological traits.

### **MAJOR ACCOMPLISHMENTS (2007–2010):**

#### Repository Collections

The Geneva germplasm repository maintains, characterizes, and distributes a collection of 1,369 grape accessions, composed of 440 wild species accessions mostly of North American origin, and 929 hybrid accessions. The hybrid accessions are predominantly “French-American hybrids” or “direct producers”, but there are historic cultivars, cultivars developed by early breeders, such as TV Munson and Rogers, and cultivars from modern breeders, most notably some 50 accessions with exceptional cold-hardiness and quality from Elmer Swenson. The collection does not contain cultivars patented in the United States. New accessions are added on an ongoing basis. Accessions are documented on the Germplasm Resource Information Network (GRIN) Web site ([www.ars-grin.gov](http://www.ars-grin.gov)), with passport data, cultivar descriptions, images, and links to data for 17 key characteristics. Significant additions to this descriptive database in 2007-2010 are:

1. SSR fingerprinting of the grape collection. 955 grape accessions were fingerprinted using 8 SSR loci and loaded into GRIN in 2008. These SSR fingerprints are being validated by fingerprinting the second vine per accession to be used as a management tool; discrepancies between the two vines per accessions will be investigated as well as redundant fingerprints when the vines are in fruit.
2. Phenology observations. In 2009 and 2010 bud break observations were taken, about 3000 in 2010. Budburst date is an important character for NE breeders who work with difficult conditions such as late frosts and relatively short growing

seasons. In 2009, bloom date was recorded for 969 accessions. These data are/will be available in GRIN.

3. Imaging. In 2008-2010, the leaves of most clonal and a few seedling accessions were imaged from both sides (~2,500 images). A number of flower clusters were imaged as well. Imaging of fruit clusters, berries, berry cross sections, and seeds has just begun. Leaf images (and flower/fruit images) are useful in taxonomy and accession identifications. Images of flowers are useful in assessing likely bunch size and architecture, and especially for male vines as it gives an idea of its contribution to the bunch architecture of offspring in crosses. The images already loaded into GRIN are available on the individual accession's page. As described below data on secondary metabolite profiles of representative accessions are being collected and virus indexing has been initiated, both in collaboration with Cornell University.

#### Phylogenomic analysis of grapes.

Even though understanding evolutionary relationships and distribution of genetic diversity is an important prerequisite to species conservation and utilization, grapevine evolutionary history has not been studied comprehensively at the DNA sequence level. We selected 304 individuals from 43 *Vitis* species and three outgroup genera drawn from across the genus' native Northern Hemisphere distribution and examined 11.44 kilobases of aligned nuclear DNA sequences from 27 unlinked gene fragments in a phylogenetic context under maximum likelihood and maximum parsimony. Networks were used to visualize conflicts. Divergence times between groups were estimated by computing Ks, the rate of silent substitution. We found substantial bootstrap support for species, and low supports for the higher-level groupings. Our results challenge current higher level classifications and provide a valuable resource for species delineation, germplasm conservation and management, and the study of economically important traits at the species level.

#### Phenotyping Secondary Metabolites of USDA-ARS Vitis Germplasm. [Collaborative Project]

Grape secondary metabolites (such as anthocyanins and other polyphenols) encompass a range of phytochemicals that are important to human nutrition and health. The distribution of these phytochemicals across a wide range of germplasm is poorly known, which limits the informed use of the germplasm for marker assisted, target oriented breeding. A collaborative project with several researchers of Cornell University was recently initiated to characterize key secondary metabolites, polyphenols in particular, of the ARS *Vitis* germplasm collections. Two years of data have been collected for 344 *V. vinifera* accessions from the Davis repository and 200 wild accessions of mainly native species from the Geneva repository. Tremendous variation of various polyphenolic compounds (anthocyanins, hydroxybenzoic acids, hydroxycinnamic acids, flavanols and flavonols) was found in the collections. This project will help develop a database of health and nutrition-related metabolites for USDA *Vitis* germplasm. It will also help develop future research models for integrating germplasm characterization with genetic and gene discovery research.

#### Screening the USDA cold-hardy grape germplasm repository for viruses and perform elimination therapy if needed. [Collaborative Project]

Considering the economic importance and broad dissemination of the Geneva grape germplasm it is important that diseases that affect cultivar vitality, productivity, and quality are absent. The virus status for almost all accessions is unknown, although a few are known to be infected with a virus. Thus, indexing the repository for economically important viruses in combination with therapy and disease elimination is of high priority. In collaboration with researchers of Cornell University, we are pursuing a project to index the entire Geneva germplasm repository for major grape viruses, identify diseased accessions and subject them to therapy elimination treatment. It is anticipated that our activities will put us in a position to deliver high-quality, virus-tested, clean propagules to stakeholders. Preliminary data from 2010 spring and fall indexing are being analyzed.

#### The National Genetic Trait Index for Grape. [Collaborative Project]

We provided support to the National Genetic Traits Index for Grape Project led by Ed Buckler. This project involved the most comprehensive genetic assessment of a USDA germplasm collection to date. The first stage of the project involved large-scale, next-generation DNA sequencing of two *V. vinifera* varieties and 7 wild *Vitis* species from the Plant Genetic Resources Unit and another eight diverse *V. vinifera* from ARS Davis, CA. From these data, over 100,000 useful genetic markers were identified. The analysis of these marker data revealed that the grapevine has maintained enormous population sizes over tens of millions of years and that linkage disequilibrium (LD) therefore breaks down extremely rapidly. These observations have important implications for the design and implementation of future genetic mapping studies in the grape and in other woody perennials crops (Myles et al. 2010). From these 100,000 genetic markers, 9,000 were selected to be assayed by a custom genotyping microarray (the Vitis9kSNP array), providing the first genome wide evaluation of genetic variation in a fruit crop. Over 2,000 *V. vinifera* and wild *Vitis* accessions were genotyped with the Vitis9kSNP array. These data have revealed several fascinating features of the domestication history and genetic structure of *V. vinifera*, including the discovery of parentage and other forms of relatedness among cultivars (Myles et al. submitted). These analyses will provide the foundation for better management and curation of the grape germplasm collection; will identify key regions of the genome that have been introgressed from wild species during the generation of cold hardy and disease resistant hybrid cultivars; will provide a high resolution phylogeny of the genus *Vitis*; and will lay the foundation for future work in marker-assisted breeding in the grapevine.

#### QTL analysis of predatory mite abundance and leaf morphology traits in a hybrid grape Population. [Collaborative Project]

We contributed to a collaborative mapping project which involved several researchers from ARS and Cornell University. Non-glandular leaf trichomes and domatia in grapes have been shown to positively influence the abundance of several species of predatory phytoseiid mites that feed on spider mite pests. These leaf structures, therefore, indirectly contribute to plant defense. Predatory mite abundance and several leaf morphological traits were measured in a grapevine population segregating for these morphological traits and regions of the genome underlying these traits was determined through QTL analysis. A genetic linkage map was constructed from 171 progeny of the cross of two interspecific hybrids, Ill. 547-1 (*V. rupestris* x *V. cinerea*) and 'Horizon', using 1,068 molecular markers. Phytoseiid mite abundance was determined for 4

years and leaf morphological traits were measured in three growing seasons. The morphological traits measured were: leaf width, leaf length, vein bristles, vein hairs, domatia size, blade bristles, and blade hairs. Domatia size is the diameter of tufts of trichomes in vein axils; bristle and hair measurements estimate the density of shorter (<0.25 mm) and more upright trichomes and longer and prostrate trichomes, respectively. A single, major QTL was identified for all years for mite abundance, domatia size, vein bristles, vein hairs, blade bristles, and blade hairs.

#### **TECHNOLOGY TRANSFER/OUTREACH:**

Distribution of grape germplasm is a primary mission of the grape repository. Since 2007, the Plant Genetic Resources Unit has distributed 9,728 grape accessions to 365 requestors. Since distributions began in 1988, the Plant Genetic Resources Unit has distributed 27,766 samples to 1,413 requestors. To assist users making informed choices the Plant Genetic Resources Unit strives to document grape germplasm accessions by collecting and uploading onto the GRIN Web site data for a diversity of descriptors that relate to chemical, disease, molecular, morphological, phonological, and production characteristics ([www.ars-grin.gov/cgi-bin/npgs/html/desclist.pl?174](http://www.ars-grin.gov/cgi-bin/npgs/html/desclist.pl?174)) as well as images of leaves, flowers, and fruit clusters. Besides the Internet, the Plant Genetic Resources Unit presents research at scientific meetings and in scientific journals. We also present our program to our primary stakeholders, the grape variety research and development community (breeders and others), and the general public during tours and field days. For example, in 2010 we contributed to the organization of the 10<sup>th</sup> International Conference on Grapevine Breeding and Genetics in Geneva, NY, and grape researchers and breeders from all over the world toured the Plant Genetic Resources Unit and the repository vineyards. We also hosted tours for the PGO (Plant Germplasm Organizational Committee) and an extended training visit by a Brazilian grape researcher. Recently, we welcomed the public during a field day.

#### **EXTERNAL SUPPORT:**

- Grape CGC funding for characterizations 2008, 2009.
- NRI- Project described above (QTL analysis of predatory mite abundance).

#### **COLLABORATORS:**

Peter Cousins, Chris Owens, and Lance Cadle-Davidson, ARS Geneva, NY; Malli Aradhya, Bernie Prins, and John Preece, ARS Davis, CA; Bruce Reisch, Jan Nyrop, Greg English-Loeb, Rebecca Loughner, Lailang Cheng, Sean Myles, and Marc Fuchs, Cornell University, Geneva and Ithaca, NY; Ed Buckler and Doreen Ware, ARS Ithaca, NY; Quinn Sinnott and Mark Bohning, ARS Beltsville, MD; the National Plant Germplasm Crop Germplasm Committee for grapes which consists of approximately 20 grape researchers and representatives from the industry, who offer expert advice and guidance pertaining to the grape Germplasm collections; and national and international grape researchers who use our collections.

#### **RECENT PUBLICATIONS:**

- Myles S, Chia J-M, Hurwitz B, Simon C, Zhong GY, Buckler E, Ware D (2010)

Rapid Genomic Characterization of the Genus *Vitis*. PLoS ONE 5: e8219

- Owens, C.L., B.I. Reisch, H.R. Schwaninger, M. Aradhya, C. J. Simon, S. Manahil, R. Loughner, K. Wentworth, G. Loeb, J. Nyrop (2010). QTL analysis of predatory mite abundance and leaf morphology traits in a hybrid grape population. 10<sup>th</sup> international conference on grapevine breeding and genetics, Geneva, NY.
- Simon, C.J., A.M. Baldo, Y. Wan (2009). Genetic Sequence Diversity In The Grape Genus (*Vitis*). Plant & Animal Genome XVII Conference, San Diego, CA.